ATTACHMENT TO RESPONSE TO OFFICE ACTION DATED NOVEMBER 18, 2003

Serial No. 09/746,921

DECLARATION OF DR. KEVIN J. THORNE

And

PATENT NO. 5525148

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.	:	09/746,921	Confirmation No. 2764
Applicants	:	Kevin J. Thorne and James J. Benedict	
Filed	:	December 22, 2000	
For	:	Composition and Process for Bone Growth and Repair	
TC/A.U.	:	1654	
Examiner	:	Patricia A. Patten	

Atty. Dkt. No.: SBI-073
Date: May 14, 2004

DECLARATION OF DR. KEVIN J. THORNE

Mail Stop: Amendments
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

PURPOSE OF DECLARATION

1. This Declaration is submitted in conjunction with a Response to the Office Action dated November 18, 2003, and is for the purpose of aiding in overcoming one or more ground of rejection or requirement made in that Office Action. This Declaration is to establish the deficiency of at least the following references as prior art against the above-identified U.S. patent application: K. Ohura et al. *J Biomed Mater Res* (1999) 44(2), 168-175; U.S. Patent No. 6,187,047 (*Kwan et al.*) and U.S. Patent No. 5,047,031 (*Constantz*), to rebut the enablement and written description rejections, and to establish unexpected results.

STATEMENT OF FACTS

2. I, Kevin J. Thorne, have earned Ph.D., M.S. and B.S. degrees in Materials Science and Engineering from the University of California at Los Angeles. I have practiced in the field of biomedical engineering for over 10 years, and am currently employed by Zimmer, Austin, Texas as a Manager of Research. In the course of such employment I am responsible for supervising and directing discovery and product development research, in addition to other duties. I have extensive knowledge and experience in the areas of materials processing, inorganic and organic polymer chemistry, osteoinductive proteins and histological bone quality assessments for medical use and for scientific research. I am familiar with the levels of education and skill that were possessed by those working in

the field of manufacture of osteogenic compositions and devices at the time of the invention which is the subject of the above-identified application. I am an inventor in the above-identified patent application and have read the specification and claims. I have also read the above-mentioned patent or literature references and understand their teachings.

3. The Examiner deems that calcium monophosphate buffers at a pH between pH 6.2 and pH 8.2. That assertion is incorrect, however, as the pKa of calcium monophosphate is approximately 4.2 and its approximate buffering range is about pH 3.2 to 5.2. Thus, the skilled practitioner would reasonably expect a composition composed of calcium monophosphate to be strongly to moderately acidic (*e.g.*, pH < 5). For reference, standard pKa values of various calcium phosphate salts are as follows:

$Ca(H_2PO_4)_2$	(monocalcium phosphate; MCP)	pKa = 4.2
$CaHPO_4{\cdot}2H_2O$	(dicalcium hydrogen phosphate; DCP)	pKa = 6.5
$3CaO{\cdot}P_2O_5{\cdot}2H_2O$	(tricalcium phosphate; TCP)	pKa = 26.0
$3.33CaO{\cdot}P_2O_5{\cdot}2H_2O$	(hydroxyapatite; HA)	pKa = 57.8
$4CaO{\cdot}P_2O_5$	(tetracalcium phosphate; TTCP)	pKa = 30.6

Reference: Prospects for the Recovery of Phosphorous from animal manures: a review. J. Greaves, P. Hobbs, D. Chadwick and P. Haygarth. Institute of Grassland and Environmental Research. North Wyke, Devon, EX20 2SB UK

4. Our discovery that pH plays a strong role in the osteogenic performance of compositions employing bone growth proteins, with "acidic environments providing dramatically superior results" (pg. 11, lines 6-9 of the Specification), was unexpected and goes against the conventional thinking at the time the invention was made.

4.1 As an example of such conventional thinking, attached to this Declaration is a copy of U.S. Patent No. 5,525,148, which teaches the customary view that the hydroxyapatite form of calcium phosphate is generally desirable in osteogenic compositions because it is the principal mineral component of teeth and bones (col. 5, lines 16-19). It is well known that hydroxyapatite is alkaline (pKa = 57.8), and yet it has historically been considered to be the osteogenic substrate of choice.

4.2 In another example from the literature, U.S. Patent No. 5,741,329, the deleterious effects of lowered pH in the vicinity of an implantable polymeric device (*i.e.*, which in some

embodiments includes bioactive compounds) are combated by maintaining relatively physiological pH surrounding the implant. In view of at least the foregoing references, it would have been considered by one of skill in the art at the time of Applicants' invention to be contrary to conventional thinking to prepare an implantable composition intended to enhance bone growth by deliberately making the composition acidic.

5. It would not have been expected by a skilled practitioner in this field at the time the present invention was made that enhanced bone formation would be achieved by the claimed acidic compositions (*i.e.*, as compared to the performance of conventional hydroxyapatite compositions or other conventional calcium phosphate-containing compositions). Figures 2-11 of the subject application demonstrate the superior performance characteristics of these acidic compositions within a wide range of calcium phosphates having different pHs.

5.1 With respect to Figures 2-5 of the subject application, one of skill in the art at the time the invention was made would have understood that the relative efficacy for *in vivo* bone formation by various calcium phosphate salt compositions containing bone growth protein (BP) could be demonstrated by measuring the explant mass, histology score, mineral concentration and mineral mass following implantation of BP containing collagen disks in *in vivo* bone formation experiments. Various calcium phosphate species (first column in Figures 2B-5B) were included in disks together with collagen/BP and tested ("CPB" in Figures 2B-5B). A control composition comprising only collagen/BP also was tested with each of the above-described samples, and its performance is reported under the heading "CB" in Figures 2B-5B). The CB control samples were used as a reference for a minimal osteoinductive response. As additional comparatives, some conventional, commercially available osteoinductive compositions (ProOsteon 200R-acidic, ProOsteon 200R-neutral, Ostite C1-C3, GB92 and Bioglass) were similarly tested. The testing protocol generally included subcutaneous implantation into rats of porous collagen (bovine tendon Type 1) disks containing a natural mixture of bovine bone growth proteins (BP). A full range of calcium phosphates was evaluated using this model, including samples with constant weight additions (50/50 wt.% particle/collagen per disk) of either monocalcium phosphate [$Ca(H_2PO_4)_2$], calcium hydrogen phosphate dihydrate [$CaHPO_4 \cdot 2H_2O$], calcium pyrophosphate [$2CaO \cdot P_2O_5$], tricalcium phosphate [α-$3CaO \cdot P_2O_5$, β-$3CaO \cdot P_2O_5$], hydroxyapatite [$3.33CaO \cdot P_2O_5(OH)_2$ (polycrystalline

and two amorphous compositions)], tetracalcium phosphate [$4CaO{\cdot}P_2O_5$] or calcium carbonate [$CaCO_3$ (aragonite), $CaCO_3$ (calcite)]. A calcium phosphate additive was considered detrimental if it either reduced the explant or mineral mass values or if it negatively influenced bone maturation.

a. Explant mass. With respect to Figures 2A-B, compared to the CB controls (*i.e.*, without calcium phosphate additives), the majority of the salt additives caused a negligible or detrimental effect on total explant mass. However, superior improvements in explant mass were observed with the addition of calcium hydrogen phosphate dihydrate [$CaHPO_4{\cdot}2H_2O$, DICAL, DCP] and calcium pyrophosphate [$2CaO{\cdot}P_2O_5$, Pyro, CP]. This latter additive essentially doubled explant mass. These experimental results indicate that explant mass values are enhanced with the use of acidic calcium phosphate additives.

b. Histology. Consistent with the explant mass results, Figures 3A-B depict superior improvements in histological score were observed with calcium hydrogen phosphate dihydrate [$CaHPO_4{\cdot}2H_2O$, DCP] and calcium pyrophosphate [$2CaO{\cdot}P_2O_5$, Pyro, CP] additives. In contrast to these data and the expectations of those skilled in the art at the time of the invention, statistically significant reductions were observed in histological score for all other salt compositions.

c. Mineral Concentration. As shown in Figures 4A-B, the mineral content was not altered by collagen samples supplemented with acidic calcium phosphate salts. Because biological tissues are produced with specific compositional profiles, a resultant deviation in mineral concentration is considered a negative osteoinductive response.

d. Mineral Mass. With respect to Figures 5A-B, statistically equivalent and enhanced improvements in mineral mass were observed with monocalcium phosphate (MCP) and calcium hydrogen phosphate dihydrate (DCP), respectively. In addition, markedly superior improvements in mineral mass were observed with calcium pyrophosphate (CP). These experimental results indicate that mineral mass values are enhanced with the use of acidic calcium phosphate additives.

5.2 With respect to Figures 6-9 of the subject application, one of skill in the art at the time the invention was made would have understood that the relative efficacy for *in vivo* bone formation by various calcium phosphate salt compositions containing bone growth protein (BP) could be demonstrated by measuring the explant mass, histology score, mineral concentration and mineral mass following implantation of BP containing collagen disks in *in vivo* bone formation experiments. Various calcium phosphate species (first column in Figures 6B-9B) were included in disks together with collagen/BP and tested ("CPB" in Figures 6B-9B). A control composition comprising collagen/BP and devitalized bone matrix also was tested with each of the above-described samples, and its performance is reported under the heading "CDB" in Figures 2B-5B). The CDB control samples were used as a reference for a strongly positive osteoinductive response. As additional comparatives, some conventional, commercially available osteoinductive compositions (ProOsteon 200R-acidic, ProOsteon 200R-neutral, Ostite C1-C3, GB92 and Bioglass) were similarly tested. The testing protocol generally included subcutaneous implantation into rats of porous collagen (bovine tendon Type 1) disks containing a natural mixture of bovine bone growth proteins (BP). A full range of calcium phosphates was evaluated using this model, including samples with constant weight additions (50/50 wt.% particle/collagen per disk) of either monocalcium phosphate [$Ca(H_2PO_4)_2$], calcium hydrogen phosphate dihydrate [$CaHPO_4 \cdot 2H_2O$], calcium pyrophosphate [$2CaO \cdot P_2O_5$], tricalcium phosphate [α-$3CaO \cdot P_2O_5$, β-$3CaO \cdot P_2O_5$], hydroxyapatite [$3.33CaO \cdot P_2O_5(OH)_2$ (polycrystalline and two amorphous compositions)], tetracalcium phosphate [$4CaO \cdot P_2O_5$] or calcium carbonate [$CaCO_3$ (aragonite), $CaCO_3$ (calcite)]. A calcium phosphate additive was considered detrimental if it either reduced the explant or mineral mass values or if it negatively influenced bone maturation.

a. Explant mass. With respect to Figures 6A,B, in comparison to the CDB controls, the majority of the salts tested had a negligible or a detrimental effect on total explant mass. Similar to the results in 2A-2B, equivalent improvements in explant mass were observed with moderately acidic calcium hydrogen phosphate dihydrate (DCP) and superior improvements in explant mass were observed with the addition of calcium pyrophosphate (CP). This additive essentially doubled explant mass.

b. Histology. Figures 7A,B show that the acidic calcium phosphates were the only additives that produced explants with equivalent histological scores equivalent to CDB positive controls. These results are consistant with those shown in Figures 3A-3B. In contrast to these data and the expectations of those skilled in the art at the time of the invention, reductions (some marked) were observed in histological score for all other salt compositions.

c. Mineral Concentration. As shown in Figures 8A-B and consistent with the results shown in Figures 4A-B, the mineral content was not altered significantly by collagen samples supplemented with acidic calcium phosphate salts. Because biological tissues are produced with specific compositional profiles, a resultant deviation in mineral concentration is considered a negative osteoinductive response.

d. Mineral Mass. With respect to Figures 9A-B, statistically equivalent and enhanced improvements in mineral mass were observed with monocalcium phosphate (MCP) and calcium hydrogen phosphate dihydrate (DCP), respectively. In addition, markedly increases in mineral mass were observed with calcium pyrophosphate (CP). Consistent with those shown in Figures 5A-B, these experimental results indicate that mineral mass values are enhanced with the use of acidic calcium phosphate additives.

5.3 Quite surprisingly, the colletive results of Figures 2A-9B indicate that osteogenic performance of bone growth protein containing compositions is enhanced by the addition of acidic calcium phosphate salts. As noted at page 13, lines 3-14 of the specification, the calcia (CaO) content and thus the Ca/P ratio in the calcium phosphate salt directly correlates with its pH, with high ratios being strongly alkaline. For example, monocalcium phosphate [$Ca(H_2PO_4)_2$] has a pH ~3.2-4.2 and dicalcium hydrogen phosphate has a pH ~5.5. The neutral transition point (pH ~7) is located with tricalcium phosphate compositions. In contrast, hydroxyapatites have a pH ~8, while tetracalcium phosphate and calcium carbonates have a pH ~10-11. Similarly surprising and inconsistent with the expectations of one of ordinary skill in the art, these results also suggest that osteogenic performance is hindered with neutral and alkaline pH additives (*e.g.* calcium phosphate salts with >2 Ca/P).

6. The conclusion that acidic calcium phosphate compositions are unexpectedly efficacious in accentuating bone protein induced bone growth, is further supported by the relative histology and

mineral mass data shown in Figures 10 and 11 (*i.e.* showing significant bone protein induced bone growth at various acidic pHs).

7. In conclusion, Applicants' observation that the majority of the calcium phosphate additives had no effect, or had a negative effect on explant mass is contrary to the expected benefits of supplementation with calcium and phosphate ion. For example, the *Ohura et al.* reference reflects the conventional line of thought at the time the present invention was made that it is the calcium and phosphorous ions that play a significant role in bone induction (page 174, col. 1 of *Ohura et al.*). The *Constantz* patent reflects the conventional view that the pH of a composition was an incidental variable dependent upon the selected cement-like, moldable or flowable structure of a calcium phosphate based composition, and that within the physiologically acceptable range, one pH was as good as another. The *Kwan et al.* reference also is consistent with the conventional view that the final, implantable osteogenic composition typically comprises hydroxyapatite (col. 5, lines 19-20). In contrast to the results discussed above, none of those references teach or suggest that acid pH compositions containing bone growth protein and a source of calcium and phosphate would have significantly enhanced osteogenic properties.

DECLARATION

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

Full name: Kevin J. Thorne, Ph.D.

Signature: Kevin J. Thorne

Date: 5/18/04